

# Belowground competition among invading detritivores

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**Abstract.** The factors regulating soil animal communities are poorly understood. Current theory favors niche complementarity and facilitation over competition as the primary forms of non-trophic interspecific interaction in soil fauna; however, competition has frequently been suggested as an important community-structuring factor in earthworms, ecosystem engineers that influence belowground processes. To date, direct evidence of competition in earthworms is lacking due to the difficulty inherent in identifying a limiting resource for saprophagous animals. In the present study, we offer the first direct evidence of interspecific competition for food in this dominant soil detritivore group by combining field observations with laboratory mesocosm experiments using <sup>13</sup>C and <sup>15</sup>N double-enriched leaf litter to track consumption patterns. In our experiments, the Asian invasive species *Amyntas hilgendorfi* was a dominant competitor for leaf litter against two European species currently invading the temperate deciduous forests in North America. This competitive advantage may account for recent invasion success of *A. hilgendorfi* in forests with established populations of European species, and we hypothesize that specific phenological differences play an important role in determining the outcome of the belowground competition. In contrast, *Eisenoides lonnbergi*, a common native species in the Eastern United States, occupied a unique trophic position with limited interactions with other species, which may contribute to its persistence in habitats dominated by invasive species. Furthermore, our results supported neither the hypothesis that facilitation occurs between species of different functional groups nor the hypothesis that species in the same group exhibit functional equivalency in C and N translocation in the soil. We propose that species identity is a more powerful approach to understand earthworm invasion and its impacts on belowground processes.

**Key words:** *Amyntas hilgendorfi*; <sup>13</sup>C and <sup>15</sup>N labeling; earthworm; *Eisenoides lonnbergi*; functional group; interspecific competition; invasive species; *Lumbricus rubellus*; *Octolasion lacteum*; stable isotope; temperate deciduous forest.

## INTRODUCTION

The importance of the belowground subsystem on ecosystem processes and properties has received increasing recognition (Wardle et al. 2004), particularly concerning invasive species (Belnap et al. 2005), land use and management (de Vries et al. 2013), and different scenarios of temperature, precipitation, and CO<sub>2</sub> concentration changes (Tylianakis et al. 2008). Belowground biota are the major drivers of decomposition (Gessner et al. 2010) and nutrient availability (Bardgett and Wardle 2010). They interact with aboveground components, affect plant productivity (Partsch et al. 2006), diversity, and community dynamics (De Deyn et al. 2003), and modulate plant

diversity effects on productivity (Eisenhauer et al. 2012) with positive and negative feedbacks between plants and soil organisms (Wardle 2006).

Belowground communities frequently have higher species diversity than the corresponding aboveground systems (Wardle 2006); however, the factors that regulate soil animal communities are poorly understood (De Deyn and Van der Putten 2005, Wardle 2006). At local scales, interspecific interactions within a trophic group, especially competition, can, in theory, play an important role in structuring communities that are resource regulated. However, most groups of soil fauna apparently coexist in high species richness with little sign of competitive exclusion (Wardle 2006). This pattern, dubbed the “enigma of soil diversity” (Anderson 1975), led to the conclusion that competition is unlikely to be a major structuring force in soil fauna communities. Instead, niche complementarity and facilitation have been proposed as the primary forms of interspecific interaction in soil fauna (Wardle 2006, Hedde et al. 2010).

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If exceptions to the “enigma of soil diversity” exist, they might occur in taxa with low species richness at local scales, such as earthworms. Earthworms (Annelida: Clitellata) are the dominant animal group and ecosystem engineers in temperate soil; their activity strongly affects all major soil ecosystem functions, including decomposition, organic matter transformation, biological regulation, and soil engineering (Turbé et al. 2010). Earthworm species richness at local scales is usually limited to up to 10–12 species (Decaëns 2010), and is frequently in the range of four to six in newly invaded habitats in the temperate region (Hale et al. 2006, Fahey et al. 2013). In this group, competitive exclusion has frequently been suggested to be the major factor structuring its communities (Chauvel et al. 1999, Decaëns 2010). However, direct evidence of competition and its mechanism is still lacking (Uvarov 2009).

Recent studies focusing on natural earthworm assemblages suggest that earthworm communities are highly structured by interspecific interactions, potentially competition (Jiménez et al. 2012). Interspecific competition has been inferred indirectly by differences in maturation rate (Lowe and Butt 2002), growth (Winsome et al. 2006), survival (Abbott 1980), and reproduction (Elvira et al. 1997). However, identification of a limiting resource is necessary to establish presence of competition. Recently, Zhang et al. (2010) proposed a mechanistic explanation in a potential case of competition in which an Asian invasive species, *Amyntas agrestis*, indirectly impedes the ability of the European species *Lumbricus rubellus* to process leaf litter by reducing soil bacteria abundance, leading to decreased litter consumption and growth. Their explanation, hereafter referred to as the “habitat modification hypothesis,” while not supporting competition for food resources, demonstrates that belowground interactions may be more complex than previously thought.

Functional groups are clusters of species playing similar roles in the same ecosystem processes; species in a functional group exhibit functional equivalency and some degree of redundancy to the system (Blondel 2003). Due to this similarity, competition is more likely within the same functional group, and coexistence of species in this case is only possible when temporal and spatial differences exist in their resource exploitation (Ritchie and Olff 1999, Voigt et al. 2007). The most commonly used functional grouping in earthworms is based upon their feeding and burrowing behaviors: epigeic species are litter feeders and leaf litter/soil surface dwellers; endogeic species are soil feeders and live predominantly in the soil; and anecic species are litter feeders that live in permanent vertical burrows extending deep into the soil (Bouché 1977). Earthworms in these categories are assumed to affect soil vertical mixing and organic matter translocation differently, and thus these functional groups have been used widely in recent studies focusing on ecosystem functions (Bohlen et al. 2004, Crumsey et al. 2013).

The ongoing invasion of *Amyntas hilgendorfi*, an Asian species native to Japan, into deciduous forests in the Mid-Atlantic region of the United States provides a unique opportunity to study interspecific interactions with both native and nonnative resident species. We combined laboratory mesocosm experiments using  $^{13}\text{C}$  and  $^{15}\text{N}$  double-enriched leaf litter and field observations to test the hypotheses that (1) species from the same functional group, especially epigeic species, compete for food, (2) facilitation occurs between species of different functional groups, (3) competition between the epigeic species is not mediated by soil microbial modification (habitat modification hypothesis), and (4) reduced litter availability due to competition for leaf litter between epigeic species lead to negative, non-additive effects on the translocation of litter-derived fresh C and N into subsurface soil.

## METHODS

### Field site

Field sampling was conducted at the Smithsonian Environmental Research Center (SERC), Edgewater, Maryland, USA (38°53'17.0" N, 76°33'14.3" W). The majority of the upland forests at SERC are composed of successional stands of different ages that were cleared in the past for agricultural uses and are now part of the tulip poplar association (Brush et al. 1980). The dominant tree species are tulip poplar (*Liriodendron tulipifera*), sweet gum (*Liquidambar styraciflua*), red maple (*Acer rubrum*), black cherry (*Prunus serotina*), box elder maple (*Acer negundo*), American beech (*Fagus grandifolia*), oaks (e.g., *Quercus falcata*, *Q. alba*), and hickories (e.g., *Carya tomentosa*, *C. glabra*) (Higman 1968). Soils at our study location have been classified as Collington sandy loam (fine-loamy mixed, active, mesic Typic Hapludult; Szlavecz et al. 2011), with a mean pH of 5.1, 32% silt, 20% clay, and 5.6% organic matter content (K. Szlavecz, unpublished data).

Earthworms can be found in all forest stands in the tulip poplar association (Szlavecz et al. 2011). Their density, biomass, and functional group dominance vary depending on location and season and reach as high as 433 individuals and 155 g (fresh mass) per square meter (Szlavecz and Csuzdi 2007, Ma et al. 2014). Earthworm assemblages at SERC are generally dominated by non-native European Lumbricidae species, such as *Lumbricus rubellus* Hoffmeister, 1843, *Lumbricus friendi* Cognetti, 1904, *Octolasion lacteum* (Orley, 1881), and *Aporrectodea caliginosa* (Savigny, 1826). In old forest stands (150+ years post-agriculture), *O. lacteum* is absent, and a native species, *Eisenoides lonnbergi* (Michaelsen, 1894), is common and sometimes dominant. In 2010, an Asian invasive earthworm, *Amyntas hilgendorfi* (Michaelsen, 1892), was first recorded in an old forest called Treefall. Within two years, *A. hilgendorfi* dominated a large portion of this forest stand (C.-H. Chang, unpublished data).

*Mesocosm experiment with isotopically enriched leaf litter*

To test our hypotheses of competition and facilitation among species of different functional groups, a laboratory experiment with different earthworm species and species combinations was conducted using  $^{13}\text{C}$  and  $^{15}\text{N}$  double-enriched leaf litter as food. Four species of earthworms were selected based on their origins and functional groups: *A. hilgendorfi*, an epigeic species of Asian origin, *L. rubellus*, an epigeic species from Europe, *O. lacteum*, an endogeic species from Europe, and *E. lonnbergi*, a native endogeic earthworm. All species (Appendix S1), were collected at SERC, and kept in the dark at 17°C and 40% relative humidity (RH).

Forest soil from a depth of 0–15 cm was collected at SERC, sieved through a 2-mm sieve, and kept moist in 19-L (5 gallon) buckets at 10°C.  $^{13}\text{C}$  and  $^{15}\text{N}$  double-enriched tulip poplar (*L. tulipifera*) leaf litter (mean  $\pm$  SE;  $\delta^{13}\text{C} = 27.10\text{‰} \pm 0.18\text{‰}$ ,  $\delta^{15}\text{N} = 890.33\text{‰} \pm 10.95\text{‰}$ ; Appendix S1) produced in Bernard et al. (2015) with petioles removed was broken by hand and sieved through a 4-mm sieve. Mesocosms consisted of 2-L white plastic containers with perforated lids (14.8  $\times$  14.9 cm; height  $\times$  diameter) to retain moisture (Snyder et al. 2013), and 1450.0 g sieved and mixed soil was added into the mesocosms. Gravimetric water content was adjusted to 38%, first by adding 85.6 mL water, then misting 43.5 mL water after spreading 4.0 g (dry mass) of enriched litter on the surface. The amount of leaf litter added equaled 233 g/m<sup>2</sup>, similar to mean autumn litter fall at SERC (K. Szlavecz, unpublished data). Mesocosms were pre-conditioned in the dark at 40% RH and 17°C for 16 d prior to the addition of earthworms.

A total of 10 treatments and one control, all with six replicates, were set up: four were single-species treatments with each of the four earthworm species and six were two-species treatments with all combinations of the four species. The control contained both soil and litter but no earthworms. Four *A. hilgendorfi*, six *L. rubellus*, 12 *O. lacteum*, and six *E. lonnbergi* individuals were added into the single-species treatments with the respective species. For the two-species treatments, two *A. hilgendorfi*, three *L. rubellus*, six *O. lacteum*, and three *E. lonnbergi* were added into the respective treatments. The numbers of individuals were chosen for each species to take into account both density in the field and individual biomass differences to mimic potential co-occurrence conditions in the field. The mean fresh biomass of individuals was ( $\pm$  SD) 2.16  $\pm$  0.19 g for *A. hilgendorfi*, 0.57  $\pm$  0.06 g for *L. rubellus*, 0.24  $\pm$  0.02 g for *O. lacteum*, and 0.86  $\pm$  0.17 g for *E. lonnbergi*. The mesocosms were incubated in the dark under 40% RH at 17°C for 21 d. Gravimetric water content was adjusted on days 1, 3, 6, 9, 12, 17, and 21 to 35%. At the end of the experiment, earthworms were removed, counted, and weighed. Leaf

litter was collected, dried at 60°C, and weighed. All soil in the mesocosm was collected and divided into the 0–5 cm layer, and a lower layer that roughly equaled 5–10 cm. Earthworm specimens were dissected to remove gut content, freeze-dried, and homogenized. Soil was sieved through a 4-mm sieve and homogenized. Subsamples of soil were stored at –20°C for microbial analysis; another subset was dried at 60°C, and ground. Earthworm and soil samples were analyzed for  $^{13}\text{C}$  and  $^{15}\text{N}$  content as described in the following section.

*Soil earthworm modification experiment*

To test the hypothesis of soil modification by *A. hilgendorfi* influencing the feeding of *L. rubellus*, a separate laboratory mesocosm experiment was set up. Details on mesocosm setup and adjustment of water content were the same as in *Mesocosm experiment with isotopically enriched leaf litter*, except that here the leaf litter was not isotopically enriched. The experiment was conducted in two phases in a manner similar to Zhang et al. (2010). In the first phase, 1500 g sieved soil and 2.5 g tulip poplar leaf litter were pre-conditioned for 5 d, after which either four *A. hilgendorfi* or six *L. rubellus* were added to each mesocosm in six replicates. The mesocosms were incubated for 16 d, and then taken apart and the worms were removed. The *A. hilgendorfi*-preconditioned soil (A-soil) from the six replicates was combined and mixed. The same procedure was followed for *L. rubellus*-preconditioned soil (L-soil). In the second phase, 12 new mesocosms were established with half of them containing 1500 g of A-soil and L-soil, respectively; 6.0 g of leaf litter and five *L. rubellus* were added to each of the 12 mesocosms. This phase of the experiment lasted 27 d. At the end the remaining leaf litter was collected, dried at 60°C and weighed, and the earthworms were counted and weighed.

*$^{13}\text{C}$  and  $^{15}\text{N}$  natural abundance of earthworms, soil, and litter*

We used natural abundance of  $^{13}\text{C}$  and  $^{15}\text{N}$  to assess dietary differences of earthworms in their natural habitat and to infer their “isotopic niches” (Newsome et al. 2007, Jackson et al. 2011). In September 2011 and in August 2013, earthworms, soil, and leaf litter were collected at the Treefall forest stand at SERC. The two sampling occasions reflected conditions before and after extensive invasion by *A. hilgendorfi*. Three 1  $\times$  1 m quadrats were randomly selected at least 15 m away from each other. After collecting all the leaf litter, earthworms were collected using electroshocking. Three 0–15 cm deep cores were taken from each quadrat using a 5 cm diameter soil corer, and divided into three 5-cm portions. For isotopic analysis, earthworms and soil were prepared as described previously. Roots and leaf litter were oven-dried and

ground. Earthworm, soil, roots, and leaf litter samples were analyzed for their  $^{13}\text{C}$  and  $^{15}\text{N}$ .

#### *Stable isotope analysis*

The C and N elemental and stable isotope composition of freeze-dried and powdered earthworm samples from the mesocosm experiment were analyzed at the Purdue Stable Isotope Research Facility (Purdue University, West Lafayette, Indiana, USA). Specifically, samples were analyzed using a Sercon (Sercon, Cheshire, UK) GSL combustion elemental analyzer interfaced to a Sercon 20-22 stable isotope ratio mass spectrometer. The C and N elemental and stable isotope composition of leaf litter and soil samples from the mesocosm experiment were analyzed at the UC Davis Stable Isotope Facility (Davis, California, USA) using either a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon) coupled with an Elementar Vario EL Cube or Micro Cube elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany), or a PDZ Europa ANCA-GSL elemental analyzer (Sercon). The C and N elemental and stable isotope composition of earthworm, leaf litter, root, and soil samples collected from the field were analyzed at the Smithsonian OUS/MCI Stable Isotope Mass Spectrometry Laboratory (Smithsonian Museum Conservation Institute, Suitland, Maryland) using a Thermo Delta V Advantage mass spectrometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA) coupled with a Costech 4010 Elemental Analyzer (Costech Analytical Technologies, Valencia, California, USA).

Stable isotope ratios of C and N ( $^{13}\text{C}/^{12}\text{C}$  and  $^{15}\text{N}/^{14}\text{N}$ ) were expressed using delta ( $\delta$ ) notation:  $\delta^{13}\text{C}_{\text{sam}}$  or  $\delta^{15}\text{N}_{\text{sam}} = [R_{\text{sam}}/R_{\text{std}} - 1] \times 1000\text{‰}$ , where  $R_{\text{sam}}$  is the isotope ratio ( $^{13}\text{C}/^{12}\text{C}$  or  $^{15}\text{N}/^{14}\text{N}$ ) in the samples, and  $R_{\text{std}}$  is the isotope ratio in the standard, which is Pee Dee Belemnite (PDB) for C and atmospheric nitrogen for N. The proportion of litter-derived C and N in earthworm tissue was estimated following Balesdent and Mariotti (1996; Appendix S2). Despite homogenization of the soils used in the mesocosms, three out of the 60 earthworm-inoculated soil samples had  $\delta^{13}\text{C}$  lower than that in the sampled initial soil at the end of the experiment, causing false “negative effects.” To accommodate this experimental condition, we excluded soil  $\delta^{13}\text{C}$  values below those of the initial soil ( $-27.44\text{‰}$ ) from the analysis, leading to exclusion of the aforementioned three samples.

#### *Phospholipid fatty acid analysis*

Phospholipid fatty acid (PLFA) analysis was used to characterize soil microbial communities. Samples were prepared and analyzed as described by Buyer and Sasser (2012), using 19:0 phosphatidylcholine (Avanti Polar Lipids, Alabaster, Alabama, USA) as an internal standard for quantitative analysis. Gas

chromatography was conducted on an Agilent 6890 gas chromatograph (Agilent Technologies, Wilmington, Delaware, USA) equipped with autosampler, split-splitless injector, and flame ionization detector. The system was controlled with MIS Sherlock (Microbial ID, Newark, Delaware) and Agilent ChemStation software. Fatty acids were identified using the PLFAD1 calibration mix and PLFAD1 peak library (Microbial ID). Random samples were run on a Clarus 500 GC-MS (Perkin-Elmer, Waltham, Massachusetts, USA) to confirm fatty acid identifications.

#### *Statistical analysis*

All statistical tests were conducted in R v3.1.2 (R Core Team 2014). For the mesocosm experiment with isotopically enriched leaf litter, one-way ANOVA followed by Tukey’s HSD (honestly significant difference) test for multiple comparisons was used to test for species treatment effects on biomass and earthworm tissue  $^{13}\text{C}$  and  $^{15}\text{N}$  abundances within each earthworm species. General linear models (GLMs) were used to investigate the effects of earthworm species and species interactions on soil  $^{13}\text{C}$  and  $^{15}\text{N}$  abundances using the biomass of each earthworm species mean between initial and final masses as independent variables. PLFA were combined into biomarker groups (Buyer and Sasser 2012). The effects of earthworm species and species interactions on Gram-positive, Gram-negative, and total bacteria PLFA biomarkers were assessed using GLMs under the same procedure.

For the soil modification experiment, Student’s  $t$  test was used to assess the effects of soil modification by *A. hilgendorfi* on *L. rubellus* biomass changes and litter consumption.

Data on  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  from field-collected soil, root, and earthworm samples were standardized using mean leaf litter (beech and oak) isotopic abundances (Klarner et al. 2014). Differences in the natural abundance of  $^{13}\text{C}$  and  $^{15}\text{N}$  of earthworm tissues were used to infer niche differences in a two-dimensional space. The standard ellipse area (SEA) was used to estimate and compare isotopic niche widths as described in Jackson et al. (2011). Compared to the convex hull (Layman et al. 2007), SEA is less sensitive to unequal or small sample sizes (Jackson et al. 2011), allowing us to make robust comparisons between species within a community. In brief, SEA with correction for small sample size ( $\text{SEA}_c$ ) was calculated directly from isotope data. A Bayesian SEA ( $\text{SEA}_b$ ) with  $10^4$  posterior draws was calculated to statistically compare isotopic niche widths and overlaps between species belonging to the same functional group (epigeic or endogeic). The analyses were conducted in SIBER (stable isotope Bayesian ellipses in R; Jackson et al. 2011) implemented in the package SIAR (Parnell et al. 2008).



## RESULTS

*Lab mesocosm experiment with  $^{13}\text{C}$ - and  $^{15}\text{N}$ -enriched leaf litter*

In 42 of the 60 mesocosms, all earthworms survived. Two mesocosms were excluded due to 50% mortality rate. Biomass of surviving individuals of *O. lacteum* was reduced in the presence of *A. hilgendorfi* ( $F_{3,20} = 9.802$ ,  $P = 0.003$ ; significant at  $P < 0.05$ ) in comparison to all other *O. lacteum* treatments; *O. lacteum* ( $P = 0.046$ ), *L. rubellus* + *O. lacteum* ( $P = 0.001$ ), and *O. lacteum* + *E. lonnbergi* ( $P < 0.001$ ). Earthworm species treatments had no effect on the biomass of *A. hilgendorfi* ( $P = 0.14$ ), *L. rubellus* ( $P = 0.27$ ), and *E. lonnbergi* ( $P = 0.59$ ).

Earthworm species treatments had significant effects on  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in tissues of *A. hilgendorfi* ( $\delta^{13}\text{C}$ ,  $F_{3,20} = 9.13$ ,  $P < 0.001$ ;  $\delta^{15}\text{N}$ ,  $F_{3,20} = 8.93$ ,  $P < 0.001$ ), *L. rubellus* ( $\delta^{13}\text{C}$ ,  $F_{3,16} = 28.08$ ,  $P < 0.001$ ;  $\delta^{15}\text{N}$ ,  $F_{3,16} = 21.38$ ,  $P < 0.001$ ), and *O. lacteum* ( $\delta^{13}\text{C}$ ,

$F_{3,20} = 28.86$ ,  $P < 0.001$ ;  $\delta^{15}\text{N}$ ,  $F_{3,20} = 37.18$ ,  $P < 0.001$ ), but not *E. lonnbergi* ( $\delta^{13}\text{C}$ ,  $F_{3,19} = 1.95$ ,  $P = 0.155$ ;  $\delta^{15}\text{N}$ ,  $F_{3,19} = 0.66$ ,  $P = 0.588$ ). For all species, the direction and relative changes were consistent between  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values. When *E. lonnbergi* was present,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values increased significantly in *A. hilgendorfi*, *L. rubellus*, and *O. lacteum* compared to those in their respective single-species treatments (Fig. 1). In both *L. rubellus* and *O. lacteum*,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values decreased with the presence of *A. hilgendorfi*, but the change was not statistically significant for  $\delta^{15}\text{N}$  values in *L. rubellus*. *L. rubellus* and *O. lacteum* had no effect on each other. *A. hilgendorfi*  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  tissue values increased under the presence of either *L. rubellus* or *O. lacteum*, although the result was only significant under *O. lacteum* (Fig. 1). The proportions of litter-derived C and N in earthworm tissues were in the range of 1.6–25.1% and 0.3–7.6%, respectively (Appendix S3). When taking the respective single-species treatment as 100%, *L. rubellus* and *O. lacteum* had 26.6% and 31.0% decrease in litter-derived

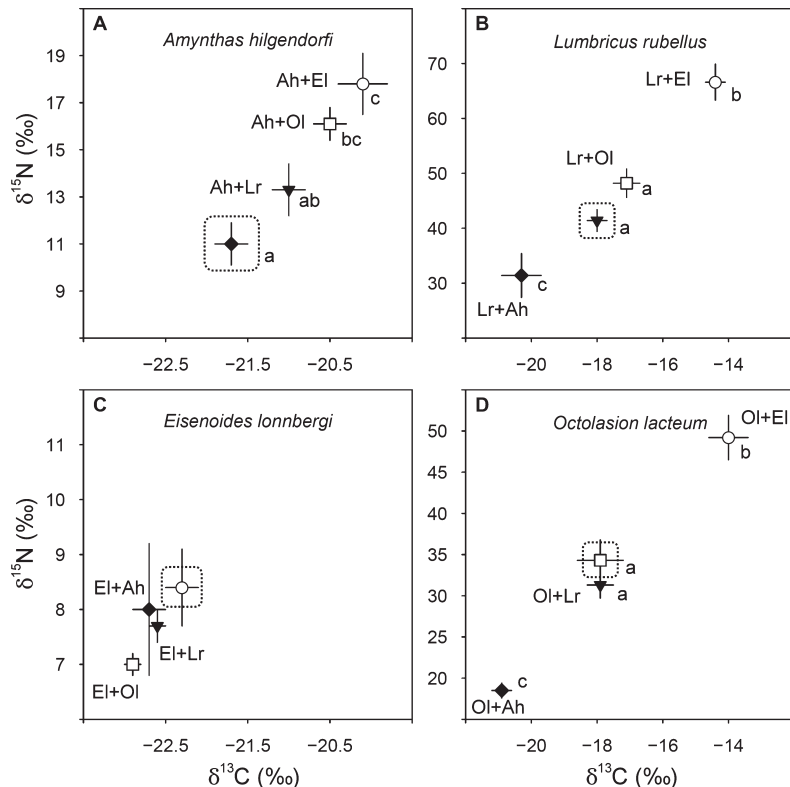


FIG. 1.  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  from (A) *Amynthes hilgendorfi* (Ah), (B) *Lumbricus rubellus* (Lr), (C) *Eisenoides lonnbergi* (El), and (D) *Octolasion lacteum* (Ol) feeding on isotopically enriched leaf litter under different species combination treatments. Symbols indicate means, error bars are SE. Single-species treatments are circled by dotted squares. For two-species treatments, the treatments are labeled next to the symbols. The same symbols are used for the same accompanying species: solid diamond, Ah; solid inverted triangle, Lr; open square, Ol; open circle, El. Within each panel,  $\delta^{13}\text{C}$  values with the same lowercase letter are not significantly different at  $P = 0.05$  (Tukey's HSD test following ANOVA); the same goes for  $\delta^{15}\text{N}$  values except for *L. rubellus* (B), in which the single-species treatment and the Lr + Ah treatments are not significantly ( $P < 0.05$ ) different from each other.

C and 23.9% and 49.2% decreases in litter-derived N in the presence of *A. hilgendorfi* (Appendix S4).

Both the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values for the mesocosm soil generally showed consistent patterns, but earthworm species effects were more evident for  $^{15}\text{N}$  because the isotopic difference between the enriched leaf litter and initial earthworm tissue was much larger for  $^{15}\text{N}$  than  $^{13}\text{C}$ . The four earthworm species showed species-specific effects on soil  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ . The native species, *E. lonnbergi*, by itself had no effect on soil  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ . *L. rubellus* increased  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in both 0–5 cm ( $\delta^{13}\text{C}$ ,  $F_{1,56} = 17.331$ ,  $P < 0.001$ ;  $\delta^{15}\text{N}$ ,  $F_{1,59} = 38.715$ ,  $P < 0.001$ ) and 5–10 cm ( $\delta^{13}\text{C}$ ,  $F_{1,56} = 9.015$ ,  $P = 0.004$ ;  $\delta^{15}\text{N}$ ,  $F_{1,59} = 13.708$ ,  $P < 0.001$ ). *O. lacteum* and *A. hilgendorfi* also significantly increased soil  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , but only in 0–5 cm ( $\delta^{13}\text{C}$ ,  $F_{1,56} = 23.991$ ,  $P < 0.001$ ;  $\delta^{15}\text{N}$ ,  $F_{1,59} = 54.614$ ,  $P < 0.001$ ) and 5–10 cm ( $\delta^{13}\text{C}$ ,  $F_{1,56} = 40.547$ ,  $P < 0.001$ ;  $\delta^{15}\text{N}$ ,  $F_{1,59} = 136.370$ ,  $P < 0.001$ ), respectively (Fig. 2, Appendix S5). Earthworm species interactions generally had no effects or positive effects on soil  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ .

However, *A. hilgendorfi*  $\times$  *L. rubellus* and *A. hilgendorfi*  $\times$  *O. lacteum* interactions had negative effects on soil  $\delta^{15}\text{N}$ , though this was only significant in the former ( $F_{1,59} = 14.970$ ,  $P < 0.001$  at 0–5 cm; Appendix S5).

*Amyntas hilgendorfi* had significant negative effects on Gram-positive ( $F_{1,57} = 4.780$ ,  $P = 0.033$ ), Gram-negative ( $F_{1,57} = 14.768$ ,  $P < 0.001$ ), and total bacteria ( $F_{1,57} = 13.153$ ,  $P < 0.001$ ) PLFA biomarkers in the 0–5 cm soil layer (Fig. 3, Appendices S6–S8). In 5–10 cm, *A. hilgendorfi*  $\times$  *L. rubellus* interaction had significant negative effect on Gram-positive ( $F_{1,58} = 5.162$ ,  $P = 0.027$ ) and total bacteria ( $F_{1,58} = 4.716$ ,  $P = 0.034$ ) PLFA; *L. rubellus*  $\times$  *E. lonnbergi* interaction had significant positive effect on Gram-negative PLFA ( $F_{1,58} = 5.138$ ,  $P = 0.027$ ) (Appendices S6–S8).

#### Soil earthworm modification experiment

Final biomass of *L. rubellus* individuals in the soil modification experiment was  $0.54 \pm 0.03$  g (mean  $\pm$  SE)

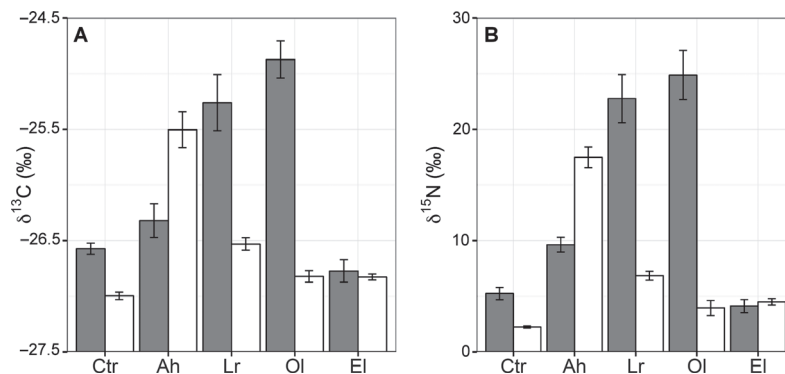


FIG. 2. (A)  $\delta^{13}\text{C}$  and (B)  $\delta^{15}\text{N}$  of bulk soil from 0–5 cm (gray bars) and 5–10 cm (open bars) in the four single-species treatments (see Fig. 1 for abbreviations) and the control (Ctr) after the 21-d experiment with isotopically enriched leaf litter. While *L. rubellus* increased  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in both depths, soils with *A. hilgendorfi* and *O. lacteum* were more enriched only in 5–10 cm and 0–5 cm, respectively. Error bars show SE.

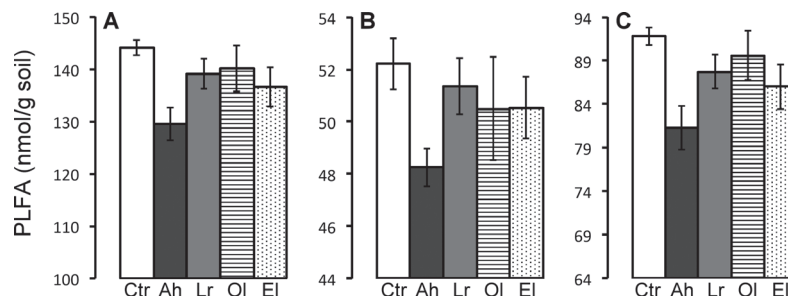


FIG. 3. Bulk soil (0–5 cm) phospholipid fatty acid (PLFA) biomarker concentrations in the single-species treatments containing *A. hilgendorfi*, *L. rubellus*, *O. lacteum*, or *E. lonnbergi* (see Fig. 1 for abbreviations) and in the control (Ctr), showing reduced PLFA biomarker concentration in the *A. hilgendorfi* treatment for (A) total bacteria, (B) Gram-positive bacteria, and (C) Gram-negative bacteria. Error bars show SE.

in A-soil and  $0.60 \pm 0.04$  g in L-soil, and leaf litter consumption per individual was  $0.57 \pm 0.05$  g and  $0.51 \pm 0.06$  g in A-soil and L-soil, respectively. Neither biomass of *L. rubellus* ( $t = -1.23$ ,  $df = 10$ ,  $P = 0.25$ ) nor litter consumption by *L. rubellus* ( $t = 0.80$ ,  $df = 10$ ,  $P = 0.44$ ) between the A-soil and L-soil treatments were significantly different.

*Background ecosystem natural abundance  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of earthworms*

Five earthworm species, *A. hilgendorfi*, *L. rubellus*, *A. caliginosa*, *O. cyaneum*, and *E. lonnbergi*, were collected at the Treefall stand at SERC. In general, the epigeic species, *L. rubellus* and *A. hilgendorfi*, had the

lowest  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values, followed by the endogeic species *A. caliginosa* and then by the other two endogeic species, *O. cyaneum* and *E. lonnbergi* (Fig. 4). All statistical comparisons were conducted for samples collected within the same year. We did not make any comparisons between the two years due to inherent differences in spatial variability of isotope signatures between the two years, potentially caused by differences in local understory vegetation. The Bayesian approach suggested that between the two epigeic species, the isotopic niche width of *A. hilgendorfi* ( $\text{SEA} = 4.82$ ) was larger than that of *L. rubellus* ( $\text{SEA} = 1.18$ ;  $P = 0.005$ ), and 23.4% of the SEA of *L. rubellus* overlapped with that of *A. hilgendorfi* (Fig. 4B). Isotopic niche widths of the three endogeic species were not

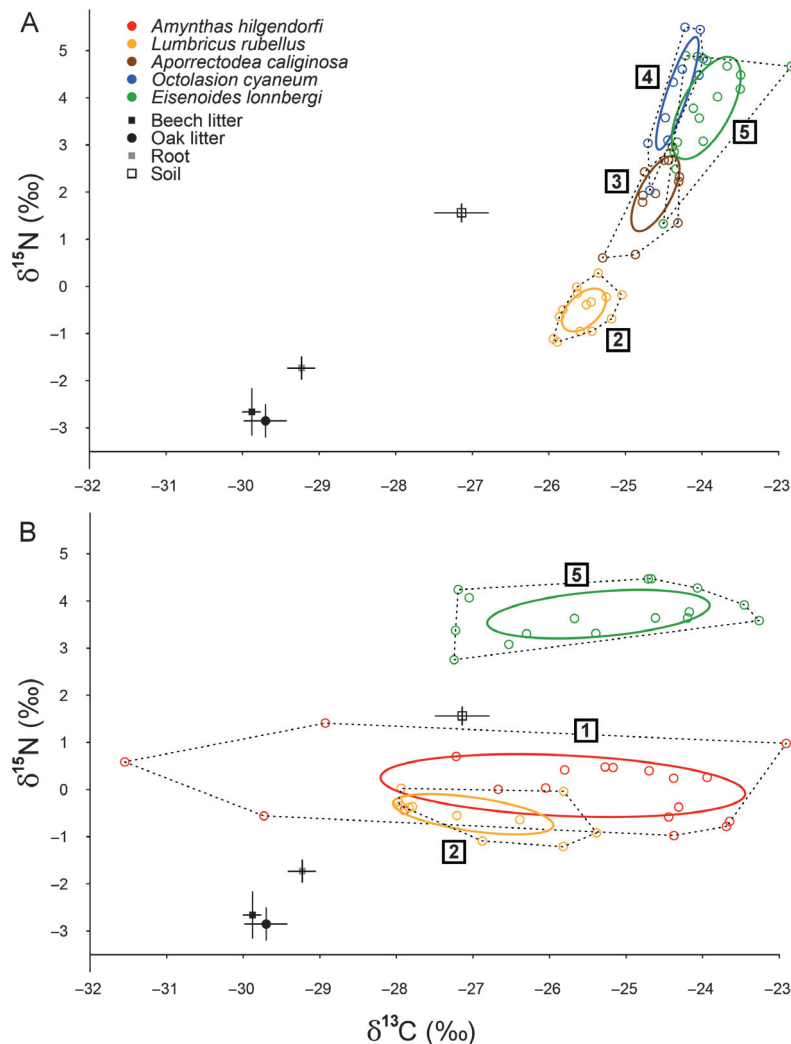


FIG. 4. Isotopic niche widths of epigeic (*A. hilgendorfi* [1] and *L. rubellus* [2]) and endogeic earthworm species (*Aporrectodea caliginosa* [3], *Octolasion cyaneum* [4], and *E. lonnbergi* [5]) inferred from the standard ellipse areas with sample size correction (SEA; colored solid lines) based on  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ . The convex hull of each species (dotted lines) was also shown for comparison. Earthworm specimens were sampled at SERC in (A) 2011 and (B) 2013. The isotope data were standardized using leaf litter (mean of beech and oak). Each colored open circle represents an earthworm individual. Values of leaf litter, roots, and soil also are presented; error bars show SE.

significantly different, and the SEA of *E. lonnbergi* overlapped with that of *O. cyaneum* by only 3.1% (Fig. 4A).

#### DISCUSSION

Using isotopically ( $^{13}\text{C}$  and  $^{15}\text{N}$ ) double-enriched leaf litter in combination with natural stable isotope abundance soil in lab mesocosms, we observed shifts in isotope ratios of earthworm tissues in paired-species treatments relative to those in single-species treatments. These shifts indicated that species altered their feeding behavior in the presence of a potential competitor by consuming more or less leaf litter than they would under intraspecific pressure. Given our knowledge of individual species' feeding preferences, the direction of the shifts in isotope ratios, and a recorded decrease in biomass of one species (*O. lacteum* in the presence of *A. hilgendorfi*), we believe that our experiments demonstrated competition for food resources among invasive earthworms.

Specifically, the results support our first hypothesis that the epigeic species, *A. hilgendorfi* and *L. rubellus*, compete for leaf litter and the latter shows reduced litter assimilation when *A. hilgendorfi* is present. Our earthworm tissue isotope data did not show any evidence of facilitation between epigeic and endogeic species and thus did not support hypothesis 2. In fact, leaf litter consumption, earthworm tissue stable isotopes, litter C and N assimilation, and fresh organic matter incorporation into soil all indicate that *O. lacteum* behaves more like an epigeic species that feeds on leaf litter and is active primarily in the 0–5 cm depth. This species experienced reduced leaf litter assimilation and even loss of biomass when *A. hilgendorfi* was present, which was similar to *L. rubellus* in the *L. rubellus* + *A. hilgendorfi* treatment.

Since leaf litter is the only primary source of isotopically enriched C and N, an increase in  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$  in the soil can be used as an indicator for incorporation of litter-derived C and N into soil organic matter due to earthworm activity. As a consequence of the reduced litter consumption by *L. rubellus* and *O. lacteum* under competitive pressure by *A. hilgendorfi*, translocation of litter-derived N into 0–5 cm soil by *L. rubellus* and *O. lacteum* is negatively affected (hypothesis 4), though the effect is only statistically significant in *L. rubellus*. Moreover, although *A. hilgendorfi* decreases soil bacteria abundance, soil modification by *A. hilgendorfi* does not affect the biomass of *L. rubellus* and its leaf litter consumption. This contradicts the prediction of the habitat modification scenario (hypothesis 3), and strengthens our hypothesis 1. Altogether, our results suggest that when competing for leaf litter against *L. rubellus* or *O. lacteum*, *A. hilgendorfi* is a superior competitor. While our findings need to be confirmed in the field, to our knowledge,

this is the first study documenting direct evidence of competition between earthworms and demonstrating food as a potentially limiting resource.

Litter quality may explain the contrasting results in our study and in the habitat modification scenario reported by Zhang et al. (2010). The oak litter used as a food source for earthworms in Zhang et al. (2010) is more recalcitrant than the tulip poplar litter, a highly palatable food source, used in our study. It is well known that slowly decomposing litter types, such as oak, need microbial preconditioning before macrodecomposers accept them as food (Lavelle 1997), which may take months in the field (Zicsi et al. 2011). If litter quality does play a role, the mechanisms through which competition takes place between *A. hilgendorfi* and *L. rubellus* will be context dependent, and likely to be determined by litter species and therefore plant community composition.

Natural isotopic abundance from SERC suggests that the isotopic niche width of *A. hilgendorfi* is about four times that of *L. rubellus*. The wide range in natural abundance  $\delta^{13}\text{C}$  values for *A. hilgendorfi* is clear evidence that the Asian invader feeds on a broad range of  $\text{C}_3$  plant litter. For an annual species that needs to grow from a cocoon to a sexually mature individual in about four months (Greiner et al. 2012), dietary flexibility is crucial to its survival, and likely contributes to its worldwide success as an invader (Zhang et al. 2010).

At this early stage of *A. hilgendorfi* invasion at SERC, it is unclear whether the new invader will outcompete *L. rubellus*, another nonnative species, most likely established a long time ago. In addition to *A. hilgendorfi* being the superior competitor for food resources, about one-fourth of the isotopic niche of *L. rubellus* overlaps with that of *A. hilgendorfi*. In the locations where the two species co-occur, density of *L. rubellus* is low when *A. hilgendorfi* becomes dominant (C.-H. Chang, *personal observation*). This evidence suggests that *A. hilgendorfi* may outcompete *L. rubellus*. However, the fundamental question is whether there are enough stabilizing niche differences between the two species to overcome fitness advantages potentially held by *A. hilgendorfi* (Chesson 2000, Adler et al. 2007, MacDougall et al. 2009). Species-specific differences in phenology play an important role if the two species do coexist. Phenology can promote stabilizing niche differences by allowing the phenologically offset species to acquire unused resources, and, in some cases, lead to fitness advantages (Godoy and Levine 2014). *A. hilgendorfi* is an annual species that overwinters as cocoons. It does not thrive at low temperature and all adult individuals die by the beginning of winter. An unusually long winter in 2014 appeared to delay the *A. hilgendorfi* life cycle by a month and led to a relatively low summer density (C.-H. Chang, *personal observation*). *L. rubellus* has a life span of several years. It is active at the



peak of litter fall (late October–early November), when most *A. hilgendorfi* have died, and it stays active throughout the winter in the Mid-Atlantic region, thus having access to abundant leaf litter resources. These species-specific traits and phenology differences between the two species may reduce the relative fitness advantage of *A. hilgendorfi* and lead to coexistence.

A central question of invasion biology is what drives the coexistence between the invader and native species (Chesson 2000, MacDougall et al. 2009). This question is especially intriguing for earthworm assemblages in Eastern deciduous forests where only a few native species, including *E. lonnbergi*, are able to maintain relatively high densities even when nonnative earthworms dominate the community. We propose that niche differences are fundamental in the continuing persistence of *E. lonnbergi* under the invasion of both European and *Amyntas* earthworms. *E. lonnbergi* is often classified as an endogeic species, yet there is negligible overlap in the isotopic niches between *E. lonnbergi* and invasive endogeic species in the field; rather, it appears to occupy a unique trophic position. Moreover, in the mixed-species experiments, *E. lonnbergi* was not affected by any of the other earthworms. Our finding supports the idea that native soil decomposers with a distinct trophic position could expand into previously unoccupied regions (Melody and Schmidt 2012).

Our results do not support the species equivalency view within a functional group in key ecological processes, and further corroborate the idea that these functional group classifications are context dependent (Neilson et al. 2000). C and N translocation into soil is viewed as the fundamental difference between epigeic and endogeic earthworms, yet our four species exhibited four different patterns of soil C and N incorporation. Moreover, the endogeic *O. lacteum* behaved most closely to what is expected of an epigeic earthworm. While previous studies suggest that this species feeds on soil (Zicsi et al. 2011) and utilizes old C resources (Ferlian et al. 2014) or soil microorganisms (Marhan and Scheu 2005), Ferlian et al. (2014) and Xia et al. (2011) also noted that plant material is important to *O. lacteum*. Here, we further demonstrated that *O. lacteum* can consume leaf litter in addition to highly decomposed plant material. During collecting earthworms in the field for our experiments, we observed close association between *O. lacteum* and understory plant roots. Fine roots have been shown to be 2–3‰ more enriched in  $\delta^{15}\text{N}$  compared to leaf litter (Pollierer et al. 2009), a difference similar to the natural isotopic abundance difference observed between *O. lacteum* and the two epigeic species in our study. Recent studies have demonstrated that rhizosphere C flux in temperate deciduous forests is more important for members of the soil food web, including *O. lacteum*, than previously expected (Pollierer et al. 2007, Gilbert et al. 2014), and

that relatively fresh C is a significant part in the nutrition of this species. The absence of roots in our mesocosms, combined with the requirement of fresh organic materials, might have caused *O. lacteum* to consume large amounts of leaf litter. Such contrasting results from different studies reveal the differences in the physiological and realized niches of *O. lacteum* and the plasticity of this species under different abiotic and biotic factors, especially food resource availability and interspecific competition. Such plasticity can have important implications for the mass as well as the chemical composition of residual surface litter and litter translocation belowground and may help explain previous observations at SERC forest sites of strong heterogeneity in litter consumption and decay chemistry (Filley et al. 2008).

This study adds to a growing body of research calling for a more cautious approach when using functional groups in studying ecological processes. For communities with relatively low species richness at local scales, species identity can be a better alternative, especially when species-specific responses are present (Fong and Fong 2014) or when species equivalency is disrupted due to natural or anthropogenic disturbance (Voigt et al. 2007), such as biological invasion. We argue that using species identity is a more powerful approach in earthworm invasion studies in temperate North America, where most earthworm communities are composed of only four to six species (e.g., Hale et al. 2006, Fahey et al. 2013) from a pool of about 10–12 European lumbricids. As belowground C and N biogeochemistry and modeling are the current focus of earthworm invasion studies in North America, incorporating species-specific information on C and N translocation and soil vertical mixing is critical for a better understanding of the processes involved.

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